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Title: Gastric cooling and menthol cause an increase in cardiac parasympathetic efferent activity in healthy adult human volunteers

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Running Title: Gastric cooling and cardiac vagal tone

Abstract: Gastric distension increases blood pressure and heart rate in young, healthy humans, but little is known about the effect of gastric stretch combined with cooling. We used a randomised crossover study to assess the cardiovascular responses to drinking 300ml of Ispaghula husk solution at either 6oC or 37oC in 9 healthy humans (age 24.08{plus minus}9.36 years) to establish the effect of gastric stretch with and without cooling. The effect of consuming peppermint oil capsules to activate cold thermoreceptors was also investigated. ECG, respiratory movements and continuous BP were recorded during a 5-minute baseline period, followed by a 115-minute post-drink period during which 5-minute epochs of data were recorded. Cardiac autonomic activity was assessed using time and frequency domain analyses of respiratory sinus arrhythmia to quantify parasympathetic autonomic activity, and QTc interval analysis to quantify sympathetic autonomic activity. Gastric stretch only caused a significant reduction in QTc interval lasting up to 15 minutes, with a concomitant but non-

significant rise in heart rate indicating an increased sympathetic cardiac tone. The additional effect of gastric cold stimulation was to significantly reduce heart rate for up to 15 minutes, elevate indicators of cardiac parasympathetic tone and eliminate the reduction in QTc interval seen with gastric stretch only. Stimulation of gastric cold thermoreceptors with menthol also caused a significant reduction in heart rate and concomitant increase in RMSSD. These findings indicate that gastric cold stimulation causes a shift in the sympathovagal balance of cardiac control towards a more parasympathetic dominant pattern.

New Findings: 1. What is the central question of this study? How do gastric stretch and gastric cooling stimuli affect cardiac autonomic control? 2. What is the main finding and its importance? Gastric stretch causes a rise in cardiac sympathetic activity. Stretch combined with cold stimulation results in an elimination of the sympathetic response to stretch and an increase in cardiac parasympathetic activity resulting in a reduction in heart rate. Gastric cold stimulation causes a shift in sympathovagal balance towards parasympathetic dominance. The cold-induced bradycardia has the potential to decrease cardiac workload which may be significant in individuals with cardiovascular pathologies.

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Short Communication

Gastric cooling and menthol cause an increase in cardiac parasympathetic efferent activity in healthy adult human volunteers

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New Findings

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cardiac parasympathetic activity resulting in a reduction in heart rate. Gastric cold stimulation causes a shift in sympathovagal balance towards parasympathetic dominance. The cold-induced bradycardia has the potential to decrease cardiac workload which may be significant in individuals with cardiovascular pathologies.

Abbreviations: Blood pressure, BP; cardiac output, CO, systolic blood pressure, SBP; diastolic blood pressure, DBP; mean arterial pressure, MAP; nucleus tractus solitarius, NTS; electrocardiogram, ECG; root mean square of successive differences, RMSSD; central nervous system, CNS; sympathetic (autonomic) nervous system, SNS; gastrointestinal tract, GIT; transient receptor potential channels, TRP; extra-cellular fluid, ECF.

Abstract

Gastric distension increases blood pressure and heart rate in young, healthy humans, but little is known about the effect of gastric stretch combined with cooling. We used a randomised crossover study to assess the cardiovascular responses to drinking 300ml of Ispaghula husk solution at either 6^{o}C or 37^{o}C in 9 healthy humans (age 24.08±9.36 years) to establish the effect of gastric stretch with and without cooling. The effect of consuming peppermint oil capsules to activate cold thermoreceptors was also investigated. ECG, respiratory movements and continuous BP were recorded during a 5-minute baseline period, followed by a 115-minute post-drink period during which 5-minute epochs of data were recorded. Cardiac autonomic activity was assessed using time and frequency domain analyses of respiratory sinus arrhythmia to quantify parasympathetic autonomic activity, and QTc interval analysis to quantify sympathetic autonomic activity. Gastric stretch only caused a significant reduction in QTc interval lasting up to 15 minutes, with a concomitant but non-significant rise in heart rate indicating an increased sympathetic cardiac tone. The additional effect of gastric cold stimulation was to significantly reduce heart rate for up to 15 minutes, elevate indicators of

cardiac parasympathetic tone and eliminate the reduction in QTc interval seen with gastric stretch only. Stimulation of gastric cold thermoreceptors with menthol also caused a significant reduction in heart rate and concomitant increase in RMSSD. These findings indicate that gastric cold stimulation causes a shift in the sympathovagal balance of cardiac control towards a more parasympathetic dominant pattern.

Introduction

Ingestion of foodstuffs are known to induce physiological effects mediated by a variety of receptors in the gastric wall and associated vasculature, such as gastric stretch receptors, thermoreceptors and osmoreceptors (Gupta et al., 1979, Villanova et al., 1997, Grundy and Scratcherd, 1989) which communicate via sensory afferents to the CNS to evoke reflex physiological activity including splanchnic vasodilation (Van Orshoven et al., 2004). A typical bolus ingestion has been shown to have varied effects on cardiovascular function. Gastric stretch has been shown to cause a vagally mediated bradycardia (Grundy and Davidson, 1981), but more recently a non-noxious gastric stretch stimulus has been shown to cause a reflex tachycardia and pressor response mediated by medullary centres (Sabbatini et al., 2017). The vascular effects of gastric stretch have also been ambiguous, with some studies demonstrating a vasoconstriction in a number of splanchnic and systemic arteries following innocuous gastric stretch (Vacca et al, 1996) and others showing a mixed sympathetic vascular efferent pattern resulting in BP variation (Rossi et al., 1998). These autonomic reflexes are likely to be initiated by TRPV4, TRPP and possibly TRPA1 stretch receptors in the stomach wall communicating via vagal afferents to the NTS (Iggo, 1957; Huang, 2004; Nilius & Owsianik, 2011). TRPM8 and TRPA1, cold receptors and cold nociceptors respectively, which are located in the mammalian gastric wall, are activated by temperatures below 25°C and also menthol (Peier et al., 2002; McKemy, 2005) and are

associated with vagally mediated afferent thermosensitivity (Zhang et al., 2004; Zhao et al., 2010). Heat-activated thermoreceptors have been shown to cause gastric wall relaxation (Villanova et al., 1997) and a reflex increase in HR via splanchnic afferents (Rozsa et al. 1988). Reflex splanchnic vasodilation associated with the gastrovascular reflex (Van Orshoven et al., 2004) mediated by the release of vasoactive intestinal hormones, and also in response to the chemical properties of food cause vasodilation and increased blood flow in the superior mesenteric artery. This reflex ensures adequate blood supply and oxygenation to the gastro-intestinal tract (GIT) following food ingestion (Seth et al., 2008). However, BP does not fall during and after a bolus ingestion due to baroreflex mediated mechanisms which increase both cardiac output (CO) and peripheral arterial resistance due to an increase in sympathetic autonomic tone which manifests as a rise in BP, increased muscle sympathetic nerve activity and elevated HR (the gastrovascular reflex) which is triggered by gastric stretch receptors (Rossi, et al., 1998; Van Orshoven et al., 2004). Further, absorption from the GIT may cause physiological consequences such as pressor effects due to a blood volume loading and reflex sympathetic activation caused by osmoreceptor stimulation in response to ECF hypo-osmolarity (Lipp et al., 2005; May & Jordan, 2011). The cardiovascular and haemodynamic effects of gastric distension after food ingestion in humans therefore serve to prevent a fall in systemic BP during and after a meal which may result from a fall in splanchnic arterial resistance (Van Orshoven et al., 2004). The gastric stretch response is mediated by TRPV4, TRPP and possibly TRPA1 stretch receptors in the stomach wall via vagal afferents to the nucleus tractus solitarius (NTS) (Huang, 2004; Nilius & Owsianik, 2011). Also located in the mammalian gastric wall are TRPM8 cold receptors which are activated by temperatures below 28°C and menthol (Peier et al., 2002; McKemy, 2005) and are associated with vagally mediated afferent thermosensitivity (Zhang et al., 2004; Zhao et al., 2010), although the presence of these receptors in the human gastric wall is to be

confirmed. These two, distinct sensation-based reflex pathways provide mechanisms to respond to stomach contents of varying characteristics, potentially working independently or interacting at either a peripheral or central level to cause changes in cardiovascular sympathovagal balance. Little has so far been published on the interaction between gastric stretch and gastric cooling responses and their combined effects on the cardiovascular system. We hypothesized that when gastric stretch is combined with cooling, the activation of gastric cold thermoreceptors may communicate vagally mediated temperature-related inputs to the nucleus tractus solitarius (NTS), probably resulting in the inhibition of the stretch induced rise in HR and BP. The aim of this study was to investigate individual GIT reflex mechanisms associated with food ingestion and the effect on cardiac function by using a bolus food ingestion which was designed to stimulate gastric stretch only, and then combine this with cold stimulation and finally by stimulating cold thermoreceptors directly without gastric stretch. To test this hypothesis, we compared the cardiovascular responses to the gastric distension due food ingestion at either 37 $^{\circ}$C and 6 $^{\circ}$C in healthy subjects using a randomised crossover study. We also assessed responses to the stimulation of gastric cold thermoreceptors only by the consumption of menthol-containing peppermint oil in the same subjects.

Methods

Ethical Approval

Ethical Approval was obtained from the University of Wolverhampton Life Sciences Ethics Committee (LSEC/201011/18) and written informed consent was obtained from each subject. Studies conformed to the standards set by the Code of Ethics of the World Medical Association (Declaration of Helsinki, 2013) and were not registered in a database.

Subjects

Nine healthy, normotensive, non-smoking adult volunteers (age 24.08±9.36 years) participated in a randomised crossover study consuming either 300ml ispaghula husk (Fybogel™, Reckitt Benckiser Healthcare (UK) Limited, UK) in water at 37°C or 6°C. Fybogel™ is a dietary fibre powder (3.5g providing 5 calories) of carbohydrates containing 85% mucilage polysaccharide (Kennedy et al., 1979) used therapeutically for constipation (Dettmar & Sykes, 2008), providing a non-invasive mechanism to induce gastric stretch without affecting gastric motility and function (McIntyre et al., 1997). The same subjects also participated in a study to determine the effect of menthol gastric stimulation on cardiovascular parameters. Subjects were instructed to avoid alcohol for 24 hours, caffeine for 12 hours, and food and drink for 2 hours prior to recording sessions. Subjects attended an initial habituation visit where they were familiarised with the protocol.

Experimental Protocol

Experiments were performed in the autonomic research laboratory at the University of Wolverhampton between 08:00 and 11:00 hours. Subjects were rested comfortably in a semi-supine position, in a quiet, temperature controlled room and asked to breathe to an audible metronome set to 0.2 Hz (12 breaths per minute). A lead II arrangement of a standard 3-lead ECG was used to record continuous ECG and a respiratory belt transducer recorded respiratory movements (UFI 1132 Pneumotrace II ^{TM}). Continuous BP was monitored using a plethysmograph (Ohmeda 2300, Finapres Medical Systems, USA) from the middle finger of the left hand resting at heart level. Data were digitised and stored using PowerLab 4/25T running Chart 5 software (ADInstruments, UK) at 1kHz sampling rate with the ECG high pass filtered at 1Hz to eliminate fluctuations in ECG baseline. BP was also evaluated at the beginning and between ECGrecording periods using a brachial electronic sphygmomanometer (Boso-medicus, Bosch+Sohn, Germany) in order to ensure the

normotensive status of the subjects and to compare with plethysmograph blood pressure values.

Following a period of stabilisation (20 minutes at least), a 5-minute baseline recording was taken. Subjects then consumed 300ml of a solution of ispaghula husk in water over a period of 2 minutes. Subjects were randomly assigned to receive a drink served at either 37 °C (body temperature) or 6 °C (cold temperature) on the first visit, and the alternate temperature drink on the second visit at least one week later. Immediately after ingestion, a continuous 20-minute recording was made, followed by alternative periods of 5 minutes rest and 5 minutes recording for the rest of the first 50 minutes and then alternating periods of 10 minutes rest and 5 minutes recording until 110 minutes after ingestion. Five-minute data recoding epochs were used in order to provide a relatively short analysis period to enable short-term changes in parameter to be seen, while also providing enough data for accurate analysis of HRV parameters and standardisation of approach across each of the recording epochs.

A further experiment was performed to investigate the effect of stimulation of gastric cold thermoreceptors only. Following a rest period and the 5-minute baseline recording, subjects consumed 2 coated capsules of peppermint oil (200mg, Boots Pharmaceuticals, UK) with 10ml of isothermic isotonic saline solution to avoid cardiac autonomic activation due to water ingestion (Brown et al., 2005, Girona et al, 2014). Post-ingestion recordings were made at the same time points as in the previous experiment. A preliminary experiment was undertaken to determine the time for the release of content from 2 coated capsules of peppermint oil. Capsules were gently stirred in 0.1M HCl at 37^{o}C in dissolution apparatus (Varian 705 DS, USA). An average time to content release of 8±1.26 minutes (n=9) was established.

Data were stored anonymously for subsequent offline analysis.

Data analysis

Offline analysis of ECG recordings was performed using the Heart Rate Variability (HRV) module within Chart 5 (ADInstruments, UK). Parameters evaluated for each 5-minute recording were HR (bpm), High Frequency Power (HF, normalised units, nu) and Root Mean Square of Successive Differences in R-R interval (RMSSD, ms) assessed from successive R-R intervals. HF power and RMSSD were used to assess cardiac parasympathetic activity (Task Force, 1996). Cardiac sympathetic activity can be assessed using analysis of ECG QT interval, where it has been demonstrated that reductions in QT are seen with pharmacological stimulation of β1 receptors acting as a sympathomimetic experimental model (Magnano et al., 2002). QT interval corrected for heart rate using the Bazett's formula (QTc) was used to assess cardiac sympathetic activity independent of heart rate (Ahnve & Vallin, 1982; Magnano et al., 2002, Bazett, 1920; Apkon & Nerbonne,1988) and was measured from 3 cardiac cycles each from the beginning, middle and end of the 5 minute recordings during the inspiratory phase of ventilation in order to eliminate the effect of parasympathetic influence on sympathovagal balance during the cardiac cycle (Badra et al., 2001).

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were averaged from beat-to-beat recordings, and mean arterial pressure (MAP) was calculated, to evaluate cardiovascular pressor effects.

Data are reported as group mean (±SD) for every 5 minute recording period. In order to assess change in parameter over the time course of the experiments, data were normalised to the individual subject baseline value and are presented as a numerical change from baseline.

Statistics

The effects of each drink over time were analysed by comparing values at each time point of the post-drink period with baseline values recorded during the 5 minutes immediately before drinking. Statistical analyses were performed on data within a treatment group using one-way ANOVA for repeated measures (GraphPad Prism v.6.02, InStat, San Diego, CA) with Bonferroni *post hoc* test. Comparisons were also made between the treatments at each time point using Student's paired t-tests (Microsoft Excel 2016).

For all tests statistical significance was assessed at either the $p<0.05$ or $p<0.01$ level.

Results

None of the subjects who ingested ispaghula solution and swallowed coated capsules of peppermint oil reported any sensation of discomfort, bloating, nausea or pain during either the consumption period or the subsequent recording period.

Baseline values of all cardiovascular parameters showed no statistical difference between the two treatment visits (table 1). Similarly, no significant changes were seen in blood pressure parameters throughout the experiment.

Parameter (mean±SD)	Ispaghula Husk, 37°C (gastric stretch only)	Ispaghula Husk, 6°C (gastric stretch and cold)	Statistical Significance
Heart rate (bpm)	67.73±7.49	67.85±5.5	NS
Systolic BP (mmHg)	118±10	118±10	NS
Diastolic BP (mmHg)	78±6	76±6	NS
RMSSD (ms)	70.64±34.94	73.36±39.88	NS
HF (nu)	74.18±5.97	74.20±13.26	NS
QTc (ms)	375.08±17.31	397.63±19.85	NS

Table 1. Baseline cardiovascular parameters in each of the gastric stretch only and gastric stretch with cold experiments recorded over a five-minute period. Statistical significance was assessed using paired Student's t-test.

Ingestion of isothermic ispaghula husk solution caused a modest but non-significant increase in HR (+1.20 ± 9.03 bpm, $p>0.1$) lasting up to 20 minutes from ingestion (Fig.1) but no change in RMSSD at any time point ($p>0.1$) (Fig.2). However, QTc was significantly reduced following isothermic ingestion for up to 10 minutes, with a maximum reduction of 16.14±29.22ms at 5 minutes ($p<0.01$) (Fig.2). In contrast, cold ispaghula ingestion induced a significant 15-minute decrease in HR (-1.81±5.52 bpm, $p< 0.05$) with a peak around 10 minutes (-2.22±5.37 bpm, $p < 0.01$) (Fig.1), while RMSSD showed a significant rise for the period up to 25 and 45 minutes respectively following ingestion (maximum rise of 18.24±47.43ms at 10 minutes, $p<0.05$) (Fig.2), which may have caused the reduction in HR. Further, cold ispaghula ingestion caused an abolition of the reduction in QTc interval that was seen with isothermic ingestion. (Fig.2). Analysis of HF power showed equivalent changes to those seen in RMSSD, with no change with warm ispaghula husk solution but a significant rise from baseline with cold solution (maximum 12.06±8.34nu at 10 minutes, $p<0.01$).

PO capsule ingestion caused a significant reduction in HR (Fig.1) and increase in RMSSD ($p<0.05$) (Fig.2) from 25-50 minutes compared with baseline. This showed a similar effect to cold ispaghula husk ingestion but with a longer onset time. The time delay compared with the cold ispaghula husk ingestion could be due to the dissolution time in vivo which may have been longer than in our preliminary in vitro experiment (around 20 minutes in vivo compared with 8 minutes in vitro), possibly due to relatively low gastric motility. PO ingestion caused no change in QTc (Fig.2), indicating that there was no effect on cardiac sympathetic activity.

No significant changes in other parameters were observed in either the body temperature or cold ispaghula husk solution or PO capsule experiments, including parameters relating to BP. These results indicate that there were no additional vascular effects of these treatments, particularly with gastric cold stimulation, which concurs with previously published observations (Lee et al., 2013).

Discussion

The effect of ingestion of isothermic ispaghula husk solution shown in these experiments was to cause a reduction in QTc interval and a modest, non-significant rise in heart rate up to 15 minutes after ingestion with no change in HF(nu) or RMSSD, indicating an increase in sympathetic efferent activity to the heart, which may be indicative of an attenuated reflex tachycardia following gastric distension (Sabbatini et al., 2017). The physiological mechanism of the gastrovascular reflex (Rossi et al., 1998) is a direct neural effect of stomach distension mediated by TRPV4, TRPP, and maybe TRPA1 stretch receptors in the stomach wall via vagal fibres afferent signals to the nucleus tractus solitarius (NTS) (Huang, 2004; Nilius & Owsianik, 2011). The gastrovascular reflex is known to cause an increase in muscle sympathetic nerve activity (Vacca et al., 1996; Rossi et al., 1998, Van Orshoven *et al*., 2004), but the reduction in QTc interval shown here could indicate a concomitant rise in cardiac sympathetic efferent activity. However, with no changes in blood pressure and only a modest rise in HR seen in our experiments, it would appear that the gastric distension caused by the volume of drink administered was not sufficient to elicit a sympathetic gastrovascular response leading to significant changes in these parameters. An advantage in the use of ispaghula husk solution in these experiments is to cause gastric stretch without a significant effect on other gastric function. While various dietary fibres have been shown to have varying effects on the rate of gastric emptying, ispaghula husk has been shown to have no effect on gastric motility (Yu, et al., 2014; McIntyre, et al., 1997).

The effect of cold ispaghula husk solution was to cause a significant reduction in HR and a significant increase in RMSSD and HFnu, indicating an increase in parasympathetic cardiac tone which opposes and dominates the sympathetic mediated stretch response. Further, the observation that there was no change in QTc interval with the cold drink indicates that the gastric stretch-induced rise in sympathetic activity was either blocked due to inactivation of the stretch receptors or negated by the rise in parasympathetic activity. The effects of cold only stimulation on the gastric wall by PO stimulation of gastric cold thermoreceptors are shown to be a rise in cardiac parasympathetic activity, which is sufficient to explain the similar effects on cardiac function to the effect of cold ispaghual husk stimulation. PO stimulation eliminated the reduction in QTc interval seen with stretch only, an effect potentially caused by a reduction in efficacy of stretch receptors, but more likely to be caused by a shift in autonomic balance towards a more parasympathetic dominant state. The effect of cold stimulation of the gastric mucosa therefore appears to be as a result of a parasympathetically mediated reflex initiated by cold thermoreceptors, potentially of the TRPM8 subtype (Peier *et al.*, 2002; McKemy, 2005; Zhang et al., 2004; Zhao et al., 2010) leading to an increase in cardiac parasympathetic activity and reduction in heart rate as well as an inhibition or blockade of the cardiac sympathetic activation seen with gastric stretch. These effects not only reverse the sympathetically mediated effects of gastric stretch, but are longer lasting despite gradual warming of the drink while in the gastrointestinal tract. As with the isothermic drink, it would appear that the stimulus in these experiments in these subjects was not sufficient to cause a significant change in blood pressure.

This study provides evidence that gastric distension alone induces an increase in SNS activity to the heart, mediating an anticipatory mechanism that plays a protective role in the maintenance of postprandial BP in the event of splanchnic vasculature vasodilation (Van Orshoven et al., 2004; Vanis et al., 2012). However, when gastric distension is combined

with gastric cold stimulus, we have shown a shift in cardiac sympathovagal balance towards predominantly parasympathetically dominated efferent activity lasting for up to 30 minutes, resulting in a lasting bradycardia. This parasympathetic dominance, corresponding decrease in HR, and the potential decrease in cardiac workload may be significant in individuals with cardiovascular pathologies.

Conflict of interest

The study authors declare no conflict of interest.

References:

Ahnve, S., & Vallin, H. (1982). Influence of heart rate and inhibition of autonomic tone on the QT interval. *Circulation*, 65, 435–9. https://doi.org/10.1161/01.CIR.65.3.435

Apkon, M., & Nerbonne, J.M. (1988). Alpha 1-adrenergic agonists selectively suppress voltage-dependent K+ current in rat ventricular myocytes. *Proceedings of the National Academy of Sciences of the United States of America*, 85, 8756-8760. https://doi.org/10.1073/pnas.85.22.8756

Badra, L.J., Cooke, W.H., Hoag, J.B., Crossman, A.A., Kuusela T.A., & Tahvanainen, K.U., Eckberg D.L. (2001). Respiratory modulation of human autonomic rhythms. *American Journal of Physiology. Heart and Circulation Physiology*, 280, H2674-H2688. https://doi.org/10.1152/ajpheart.2001.280.6.H2674

Brown, C.M., Barberini, A.G., Dulloo, A.G., & Montani, J.P. (2005). Cardiovascular responses to water drinking: does osmolality play a role? *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 289, R1687-R1692. https://doi.org/10.1152/ajpregu.00205.2005

Bazett, H.C. (1920). An analysis of the time-relations of electrocardiograms. *Heart*, 7, 353-70. https://doi.org/10.1111/j.1542-474X.1997.tb00325.x

Dettmar, P.W. & Sykes, J. (2008). A Multi-centre, General Practice Comparison of Ispaghula Husk with Lactulose and Other Laxatives in the Treatment of Simple Constipation. *Current Medical Research and Opinion*, 14, 227-233. https://doi.org/10.1185/03007999809113363

Girona, M., Grasser, E.K., Dulloo, A.G., & Montani, J.P. (2014). Cardiovascular and metabolic responses to tap water ingestion in young humans: does the water temperature

matter? *Acta physiologica (Oxford, England)*, 21, 358-370. https://doi.org/10.1111/apha.12290

Grundy, D., & Davison, J.S. (1981). Cardiovascular Changes Elicited By Vagal Gastric Afferents In The Rat. *Quarterly Journal of Experimental Physiology*, 66, 307-310. https://doi.org/10.1113/expphysiol.1981.sp002560

Grundy, D., & Scratcherd, T. (1989). Sensory afferents from the gastrointestinal tract. Handbook of Physiology: The Gastrointestinal System, Motility and Circulation (2nd ed.), American Physiological Society, USA. https://doi.org/10.1002/cphy.cp060116

Gupta, B.N., Nier, K., & Hensel, H. (1979). Cold-sensitive afferents from the abdomen. *Pflugers Archiv*, 380, 203-204. https://doi.org/10.1007/BF00582158

Huang, C-L. (2004). The transient receptor potential superfamily of ion channels. *The Journal of the American Society of Nephrology*, 15, 1690–1699. https://doi.org/10.1097/01.ASN.0000129115.69395.65

Iggo, A. (1957). Gastrointestinal tension receptors with unmyelinated afferent fibres in the vagus of the cat. *Quarterly Journal of Experimental Physiology*, 42, 130-143. https://doi.org/10.1113/expphysiol.1957.sp001228

Kennedy, J.F., Sandhu, J.S. & Southgate, D.A.T. (1979). Structural data for the carbohydrate of ispaghula husk *ex Plantago ovata* forsk. *Carbohydrate Research*, 75, 265-274. https://doi.org/10.1016/S0008-6215(00)84646-7

Lee, J.K.W., Yeo, Z.W., Nio, A.Q.X., Koh, A.C.H., Teo, Y.S., Goh, L.F. & Byrne, C. (2013). Cold Attenuates Heat Strain During Work-Rest Cycles. International Journal of Sports Medicine, 34, 1037-1042. http://dx.doi.org/ 10.1055/s-0033-1337906

Lipp, A., Tank, J., Franke, G., Arnold, G., Luft, F.C., & Jordan, J. (2005). Osmosensitive mechanisms contribute to the water drinking-induced pressor response in humans. *Neurology*, 65, 905-7. https://doi.org/10.1212/01.wnl.0000176060.90959.36

Magnano, A.R., Holleran, S., Ramakrishnan, R., Reiffel, J.A., & Bloomfield, D.M. (2002). Autonomic Nervous System Influences on QT Interval in Normal Subjects. *Journal of the American College of Cardiology*, 39, 1820-6. https://doi.org/10.1016/S0735-1097(02)01852-1

May, M. & Jordan, J. (2011). The osmopressor response to water drinking. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 300, R40-R46. https://doi.org/10.1152/ajpregu.00544.2010

McIntyre, A., Vincent, R.M., Perkins, A.C. & Spiller, R.C. (1997). Effect of bran, ispaghula, and inert plastic particles on gastric emptying and small bowel transit in humans: the role of physical factors. *Gut*, 40, 223-227. https://dx.doi.org/10.1136/gut.40.2.223

McKemy, D.D. (2005). How cold is it? TRPM8 and TRPA1 in the molecular logic of cold sensation. *Molecular Pain*, 1, 16-23. https://dx.doi.org/10.1186%2F1744-8069-1-16

Nilius, B. & Owsianik, G. (2011). The transient receptor potential family of ion channels. *Genome biology*, 12, 218. https://dx.doi.org/10.1186%2Fgb-2011-12-3-218

Peier, A.M., Moqrich, A., Hergarden, A.C., Reeve, A.J., Andersson, D.A., Story, G.M., … Patapoutian, A. (2002). A TRP channel that senses cold stimuli and menthol. *Cell*, 108, 705-715. https://doi.org/10.1016/S0092-8674(02)00652-9

Rossi, P., Andriesse, G.L., Oey, P.L., Wieneke, G.H., Roelofs, J.M., Akkermans, L.M. (1998). Stomach distension increases efferent muscle sympathetic nerve activity and blood

pressure in healthy humans. *Journal of the Neurological Sciences*, 161, 148-155. https://doi.org/10.1016/S0022-510X(98)00276-7

Rozsa, Z., Mattila, J., & Jacobson, E.D. (1988). Substance P mediates a gastrointestinal thermoreflex in rats. *Gastroenterology*, 95, 265-76. http://dx.doi.org/10.1016/0016-5085(88)90480-5

Sabbatini, M., Grossini, E., Molinari, C., Mary, D.A., Vacca, G., & Cannas, M. (2017). Gastric distension causes changes in heart rate and arterial blood pressure by affecting the crosstalk between vagal and splanchnic systems in anesthetised rats. *Experimental Brain Research*, 235, 1081-1095. https://doi.org/10.1007/s00221-016-4819-x

Seth, H., Sandblom, E., Holmgren, S., & Axelsson, M. (2008). Effects of gastric distension on the cardiovascular system in rainbow trout (Oncorhynchus mykiss). *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, 294, R1648-R1656. https://doi.org/10.1152/ajpregu.00900.2007

Task force of European Society of Cardiology and North American Society of Pacing and Electrophysiology (1996). Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Circulation*, 93, 1043–1065. https://doi.org/10.1161/01.CIR.93.5.1043

Vacca, G., Mary, D.A., Battaglia, A., Grossini, E., & Molinari, C. (1996). The effect of distension of the stomach on peripheral blood flow in anaesthetized pigs. *Experimental Physiology*, 81, 385-96. https://doi.org/10.1113/expphysiol.1996.sp003943

Van Orshoven, N.P., Oey, P.L., van Schelven, L.J., Roelofs, J.M., Jansen, P.A., & Akkermans, L.M. (2004). Effect of gastric distension on cardiovascular parameters:

gastrovascular reflex is attenuated in the elderly. *Journal of Physiology*, 555, 573-83. https://dx.doi.org/10.1113%2Fjphysiol.2003.056580

Vanis, L., Gentilcore, D., Lange, K., Gilja, O.H., Rigda, R.S., Trahair, L.G., … Jones, K.L. (2012). Effects of variations in intragastric volume on blood pressure and splanchnic blood flow during intraduodenal glucose infusion in healthy older subjects. *American Journal of Physiology: Regulatory, Integrative, and Comparative Physiology*, 302, R391–R399. https://doi.org/10.1152/ajpregu.00464.2011

Villanova, N., Azpiroz, F., & Malagelada, J-R. (1997). Perception and gut reflexes induced by stimulation of gastrointestinal thermoreceptors in humans. *Journal of Physiology*, 502, 215-222. https://doi.org/10.1111/j.1469-7793.1997.215bl.x

Yu, K., Ke, M.-Y., Li, W.-H., Zhang, S.-Q. & Fang, X.-C. (2014). The Impact of Soluble Dietary Fibre on Gastric Emptying, Postprandial Blood Glucose and Insulin in Patients with Type 2 Diabetes. Asia Pacific Journal of Clinical Nutrition, 23, 210-218. http://dx.doi.org/10.6133/apjcn.2014.23.2.01

Zhang, L., Jones, S., Brody, K., Costa, M., & Brookes, S.J. (2004). Thermosensitive transient receptor potential channels in vagal afferent neurons of the mouse. *American Journal of Physiology*, 286, G983-G991. https://doi.org/10.1152/ajpgi.00441.2003

Zhao, H., Sprunger, L.K., & Simasko, S.M. (2010). Expression of transient receptor potential channels and two-pore potassium channels in subtypes of vagal afferent neurons in rat. *American Journal of Physiology*, 298, G212-G221. https://doi.org/10.1152/ajpgi.00396.2009

Additional Information

Author Contributions:

1. Conception or design of the work (PAB)
2. Acquisition, analysis, or interpretation of data for the work (L-CK, JF, PAB)
3. Drafting of the work or revising it critically for important intellectual content (L-CK, JF, PAB)
4. Approval of the final version of the manuscript (L-CK, JF, PAB)
5. Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (L-CK, JF, PAB)
6. Confirm that all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed (L-CK, JF, PAB)

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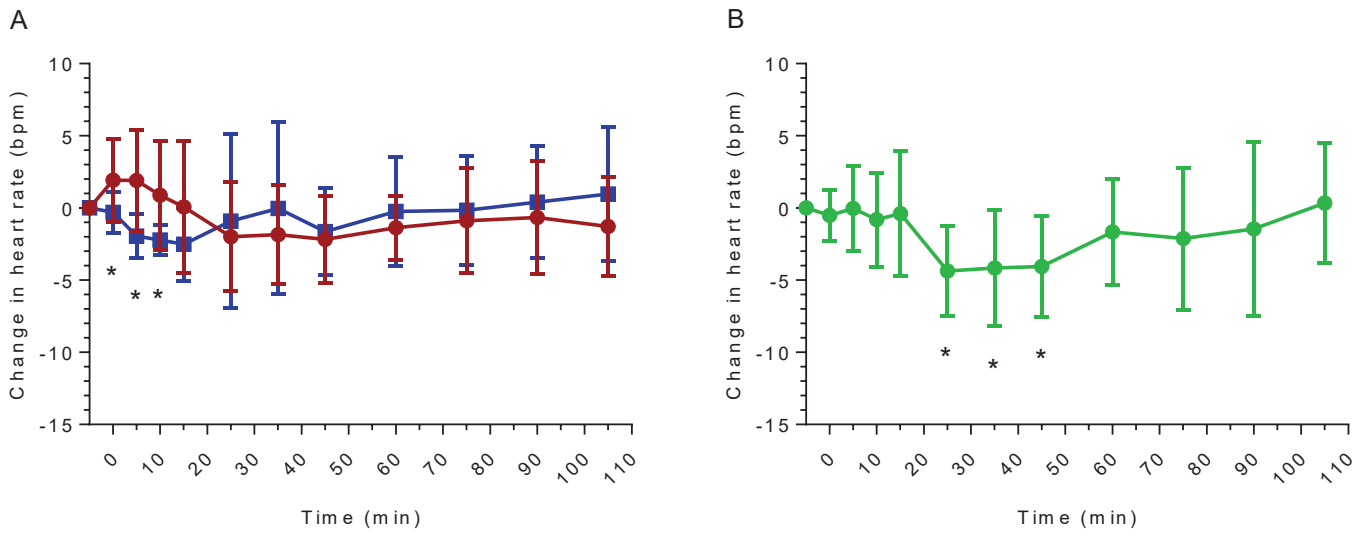

Figure 1. Time course of the changes in HR (bpm) from respective baseline values for A body temperature (gastric stretch only (red circles)) or cold ispaghula (gastric stretch and cold (blue squares)) solution ingestion and B following coated PO capsule ingestion.

Data were obtained over 5 minute intervals and are expressed as mean (±SD). Significance level was set at P <0.05. * significant difference compared with baseline.

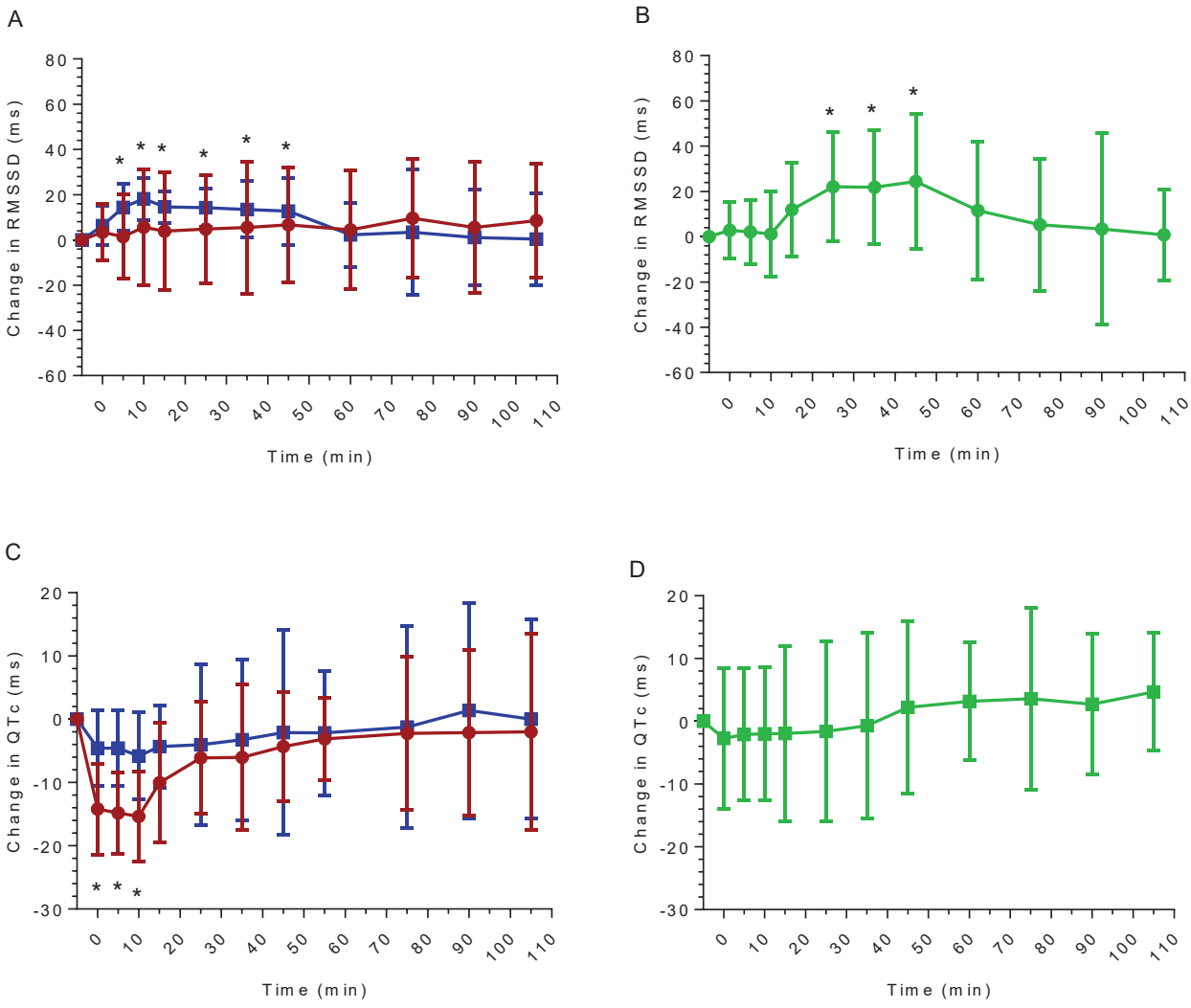

Figure 2. Time course of changes in autonomic measures. Parasympathetic changes: RMSSD (ms) from respective baseline values for A body temperature (gastric stretch only (red circles)) or cold ispaghula (gastric stretch and cold (blue squares)) solution ingestion and B following coated PO capsule ingestion. Sympathetic changes: QTc interval (ms) from respective baseline values for C body temperature (gastric stretch only (red circles)) or cold ispaghula (gastric stretch and cold (blue squares)) solution ingestion and D following coated PO capsule ingestion.
Data were obtained over 5 minute intervals and are expressed as mean (±SD). Significance level was set at P <0.05. * significant difference compared with baseline.